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PARTIAL PURIFICATION OF HOG KIDNEY SODIUM-D-GLUCOSE COTRANSPORT SYSTEM BY AFFINITY CHROMATOGRAPHY ON A PHLORIZIN POLYMER

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Summary

A brush border membrane fraction isolated from hog kidney cortex was solubilized with 0.5% Triton X-100 and subjected to affinity chromatography on a phlorizin polymer. As demonstrated by transport studies with reconstituted proteoliposomes, the polymer adsorbs the sodium-dependent D-glucose transport system. The latter can be eluted from the polymer by 0.5 M D-glucose. The purified fraction contains 0.4% of the membrane protein extract and exhibits a 20—30-fold higher transport activity than the crude membrane extract. Other brush border membrane proteins such as alkaline phosphatase and aminopeptidase M are markedly reduced in the purified fraction.

Thus, affinity chromatography on a phlorizin polymer is a suitable tool for the isolation of the sodium-glucose transport system present in brush border membranes.

Introduction

D-Glucose transport across the renal and intestinal brush border membranes is facilitated and energized by a sodium-glucose cotransport system [14]. Several authors have demonstrated recently that the sodium-glucose cotransport system can be extracted from the brush border membrane and can be incorporated into liposomes. In proteoliposomes it exhibits its typical properties such

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

as phlorizin sensitivity, sodium dependence and electrogenicity [1–4]. Attempts to purify the system have been following mainly two approaches. One is the 'negative extraction', where all other membrane proteins except the transport protein are removed from the membrane [5]. The other is a 'positive extraction', where the membrane is solubilized by detergents and a separation of the transport system from the various solubilized proteins is attempted [3].

This paper describes the partial purification of the sodium-glucose cotransport system from hog kidney brush border membranes by affinity chromatography on a phlorizin polymer. A protein fraction bound to the polymer and eluted by D-glucose shows a 30-fold higher transport activity when incorporated into liposomes than the brush border membrane. Other brush border proteins are markedly decreased in this fraction. These results indicate that affinity chromatography on phlorizin polymers is a promising tool to isolate the sodium-glucose cotransport system and perhaps other glucose transport systems.

Materials and Methods

Preparation of brush border membranes from hog kidney cortex

Pig kidneys were obtained in a local slaughterhouse immediately after killing of the animals. The kidneys were placed into ice-cold Ringer's solution (140 mM Na^+, 4.0 mM K^+, 1 mM Ca^{2+}, 146 mM Cl^-) and then perfused with the same solution to remove as much blood as possible. Slices of the kidney cortex were prepared and stored at −70°C for several weeks. Usually, up to eight kidneys were processed in one experiment.

For membrane isolation, the kidney slices were thawed at 22°C in buffer (10 mM mannitol, 2 mM Tris-HCl, pH 7.1) and the membranes were isolated according to the method of Vanier et al. [6] as modified in our laboratory [7]. The final membrane preparation was enriched about 10-fold in brush border membrane marker enzymes such as alkaline phosphatase, aminopeptidase M and trehalase. Marker enzymes for other cellular organelles such as mitochondria, endoplasmic reticulum and basolateral plasma membranes were not enriched in the membrane fraction. Up to approx. 4 g of brush border membrane protein were recovered from the eight kidneys used in each experiment.

Solubilization of membrane proteins

Membranes were suspended in solubilization buffer (100 mM NaCl, 0.5 mM EDTA (sodium salt), 10 mM Hepes, 0.2 mM dithiothreitol (pH adjusted to 7.4 with Tris)), mixed with an equal volume of 0.5% Triton X-100 in the same buffer, and incubated with stirring at 4°C for 30 min. The ratio of detergent to protein was 4 : 1 (w/w). The membrane suspension was then centrifuged for 60 min at 100 000 × *g* and 4°C; the supernatant (membrane extract AS-I-0) was used for purification of the sodium-glucose cotransport system.

Affinity chromatography

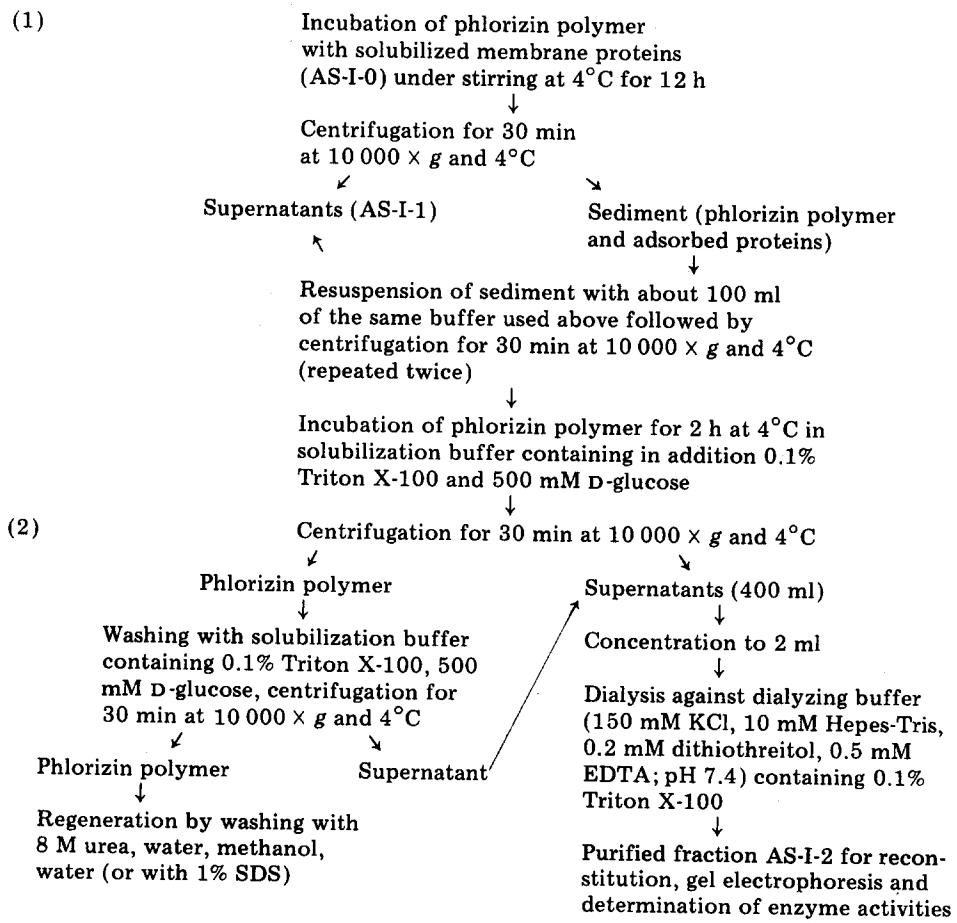
Preparation of phlorizin polymer. With 37% HCHO solution and urea,

phlorizin copolymerizes to a white resin. The preparation of this polymer and its binding capacity for lectins is described elsewhere [8]. After grinding the resin to a particle size of about 200 mesh and removing nonpolymerized compounds with 20% ethanol/water, the polymer powder was ready for use in affinity chromatography.

Affinity chromatography of brush border proteins. About 40 g of dry phlorizin polymer were equilibrated with about 100 ml of solubilization buffer containing in addition 0.5% Triton X-100, 20 mM phosphate and 500 mM mannitol. Before the membrane extract (5000 ml) was added to the polymer, the buffer solution was decanted. The mixture was then stirred for 12 h at 4°C. The subsequent steps are summarized in Scheme I.

Reconstitution of protein into liposomes

Asolectin used for reconstitution was purified in the following way. Asolectin was dissolved in petroleum ether 40 (ligroin) and acetone was added until the solution became turbid. The solution was kept at −30°C for 2 days. The precipitate was separated from the supernatant and washed again with a



Scheme I. Schematic representation of affinity chromatography.

small volume of cold petroleum ether and dried at room temperature under 0.15 mmHg vacuum. The dry lipid was then kept in $CHCl_3$ solution at −20°C under N_2. For the preparation of liposomes, the dialysis method of Kagawa and Racker [19] was used with some modifications. The $CHCl_3$ solution was evaporated at 40°C under vacuum (at least 0.1 mmHg). The dry lipid was then dissolved in dialyzing buffer containing 1% octyl-β-glucoside, which was prepared in our laboratory [9]. The protein fractions, AS-I-0, AS-I-1 and AS-I-2 were put into dialysis tubes and concentrated using 20% poly(ethylene glycol)-(M_r 40 000) dissolved in dialyzing buffer at 4°C. The concentrating process was continued until the liquid inside of the tubes was almost completely withdrawn. Asolectin solubilized in 1% octyl-β-glucoside was then added to the dialysis tube. The mixture of protein, lipid and detergent was then dialyzed against dialyzing buffer. In order to remove the detergent completely, a suitable amount of activated Biobead SM 2 was added to the dialyzing buffer in the last step of dialysis. During the whole dialysis process (40 h), dialyzing buffer was replaced five to six times. The initial lipid-to-protein ratio (w/w) was kept in the range of 20 : 1 or 40 : 1. The proteoliposomes formed during the dialysis were used directly for transport experiments. Proteoliposomes from the various protein fractions were formed simultaneously under identical conditions.

Transport studies

The uptake of D-[^{3}H]glucose by membrane vesicles and proteoliposomes was studied using the rapid filtration technique described previously [1]. 0.2 μm pore-size membrane filters (GSWP, Millipore) and 0.8 kg/cm^2 vacuum were used for proteoliposomes, and 0.6 μm pore-size membrnae filters (Type SM 11305, Sartorius, Göttingen) and 0.5 kg/cm^2 were used for membrane vesicles. The conditions for each uptake experiment are given in the legends to the figures. The transport experiments were performed at 25°C in triplicate.

Chemical determinations and enzyme assays

The protein content of the samples was determined by using the method of Lowry et al. [10] with the following modifications. (1) About 100 μl of sample were treated with 1 ml of 10% trichloroacetic acid and the precipitate was spun down. (2) Sodium dodecyl sulfate (SDS) was added to the precipitate (final SDS concentration 1%); a standard curve using bovine serum albumin was prepared in the same way and served as a reference. Lipid P_i was determined according to the method of Kinne and Faust [1] and that of Fiske and SubbaRow [11] after incineration with $Mg(NO_3)_2$ in a platinum crucible [12].

Alkaline phosphatase (EC 3.1.3.1) and aminopeptidase M (EC 3.4.1.2) activities were measured as described by Haase et al. [13]. For alkaline phosphatase determination, $MgCl_2$ (0.5 mM final concentration) and $ZnCl_2$ (1.25 mM final concentration) were added to the sample when EDTA had been used during the purification.

Enzyme and protein determinations were performed with two different amounts of the sample in duplicate.

SDS-polyacrylamide gel electrophoresis

The protein pattern of the fractions obained during the purification steps was analyzed on SDS-polyacrylamide gels (4%/30% from Pharmacia, Sweden). If there is no additional description in the legends to the figures, the recommendations from Pharmacia for running 4%/30% gels in SDS were followed. Electrophoresis buffer: 0.04 M Tris, 0.02 M sodium acetate, 2 mM EDTA and 0.1% SDS, pH 7.4. Sample buffer: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

Pre-electrophoresis without protein was performed at 70 V for 1 h. Protein fixation and SDS removal were performed electrophoretically in a 25% isopropanol/10% acetic acid solution at 24 V for 30 min. Either 0.02% Coomassie blue R or Aminoblack B in 7% acetic acid and 20% ethanol were used for staining of the protein bands. Destaining was performed in 7% acetic acid.

Prior to electrophoresis, the protein samples were incubated with 4% SDS and 2.5% mercaptoethanol in the sample buffer at 37°C overnight, or at 60°C for 15 min.

Materials

All chemicals used in this study were of the highest purity available; Triton X-100 was obtained from Serva Feinbiochemica, Heidelberg, F.R.G. and D-[^{3}H]glucose (spec. act. 15 Ci/mmol) was purchased from New England Nuclear, Dreieich, F.R.G. For centrifugation, Beckman J 21C and L 70 centrifuges were used, the indicated *g* values represent the maximum *g* values at the bottom of the tube.

Results

Transport properties of isolated hog kidney brush border membrane vesicles

The general characteristics of D-glucose transport by hog kidney brush border membrane vesicles are compiled in Figs. 1 and 2. The initial uptake of D-glucose is stimulated by a sodium gradient. The uptake shows an overshoot phenomenon and is inhibited by phlorizin. The apparent kinetic constants determined under sodium gradient conditions are a K_m value of 1.3 mM for D-glucose (Fig. 2a) and a K_i value of $1.2 \cdot 10^{-6}$ M for phlorizin. These results are similar to those reported for rat and rabbit brush border membranes [14].

Affinity chromatography of the membrane extract

Protein and enzyme composition. The membrane extract contains 82% of the membrane protein, 91% of alkaline phosphatase activity, and 93% of aminopeptidase M activity. As shown in Table I, 80% of the membrane protein, 75% of the alkaline phosphatase and 70% of the aminopeptidase M do not associate with the phlorizin polymer. The protein fraction eluted by 0.5 M D-glucose represents 0.4% of the original protein; 0.25% of alkaline phosphatase activity and 0.1% of aminopeptidase M activity are recovered in this fraction. Accordingly, the specific activity of these enzymes is lower than in the starting material. In Fig. 3, the protein pattern of three different fractions as obtained with polyacrylamide gel electrophoresis is shown. It is evident that the purified fraction still contains a large amount of protein detectable also in the crude membrane extract. However, as indicated by the arrow, one

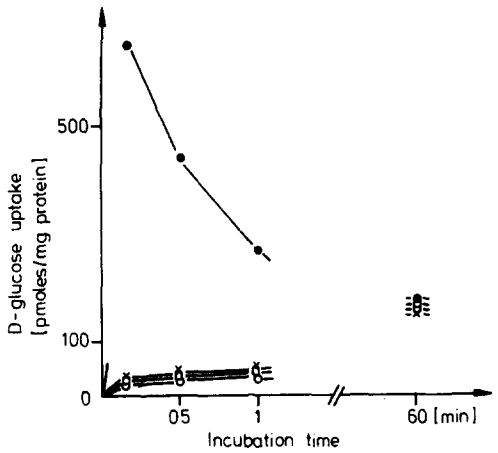


Fig. 1. D-Glucose transport by hog kidney brush border membranes: sodium dependence and phlorizin sensitivity. Brush border membranes containing 100 mM mannitol and 20 mM Hepes-Tris (pH 7.4) were incubated in the same medium containing in addition 0.1 mM D-[^{3}H]glucose and 100 mM NaSCN (●———●), 100 mM KSCN (□———□) or 100 mM NaSCN and 0.2 mM phlorizin (○———○). The points represent mean values from three experiments.

protein band corresponding to a molecular weight of about 60 000–70 000 is particularly enriched in the purified fraction. In view of the low content of the glucose transport protein in the brush border membrane [14] (0.1% of total protein) and of an enrichment of about 30-fold (see below), the expected contribution of the transport protein to the total protein would be 3%, a fact reflected well in the gel pattern.

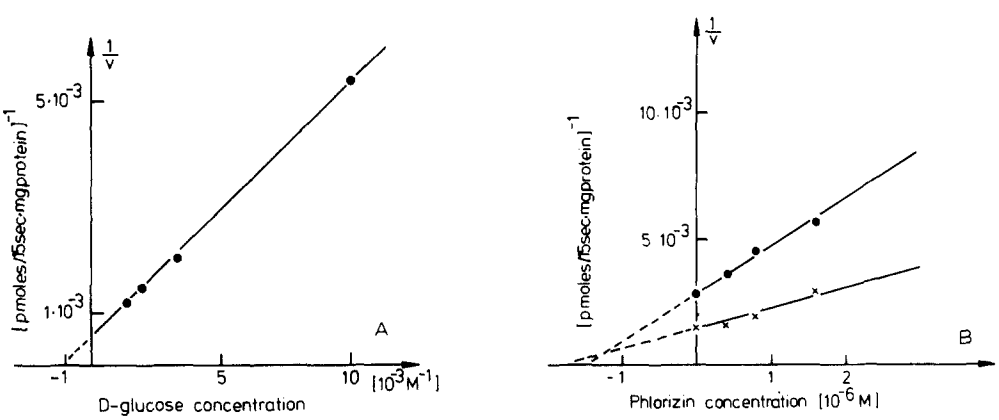


Fig. 2. D-Glucose transport by hog kidney brush border membranes: Affinity for D-glucose and phlorizin. (A) Lineweaver-Burk plot for the determination of the apparent K_m value for D-glucose. Uptake of D-glucose after 15 s of incubation in the presence of varying D-glucose concentrations but with a constant 100 mM NaSCN gradient is plotted against the D-glucose concentration. Other conditions as described in the legend to Fig. 1. (B) Dixon plot for the determination of the apparent K_i value for phlorizin. Uptake of D-glucose after 15 s of incubation in the presence of varying phlorizin concentrations was determined in the presence of a 100 mM NaSCN gradient and 0.2 or 0.5 mM D-glucose. Other conditions as described in the legend to Fig. 1.

TABLE I

BEHAVIOUR OF PROTEIN, ENZYME ACTIVITIES AND SODIUM-DEPENDENT GLUCOSE TRANSPORT DURING AFFINITY CHROMATOGRAPHY ON PHLORIZIN POLYMER

The values represent mean values from three runs of affinity chromatography. On the first line, the enrichment is given, on the second, the recovery. For the enzymes, the brush border membrane activities were taken as references; for transport, the activity in AS-I-0 was taken as reference. The specific activities of enzymes in the brush border membranes were: alkaline phosphatase, 2000 mU/mg protein and aminopeptidase M, 1700 mU/mg protein. For glucose transport, the activity found in AS-I-0 amounted to 225 pmol/30 s per mg protein. For the preparation of proteoliposomes, a lipid-to-protein ratio of 40—46 : 1 (w/w) was used. The sodium-dependent D-glucose uptake of brush border membrane was 350 pmol/30 s per mg protein.

Fraction	Protein	Alkaline phosphatase	Amino-peptidase M	Na^+-dependent D-glucose uptake
Membrane		1.0	1.0	—
	(100%)	(100%)	(100%)	
AS-I-0		1.10	1.13	1
	(82%)	(91%)	(93%)	(100%)
AS-I-1		0.94	0.87	0
	(80%)	(75%)	(70%)	
AS-I-2		0.65	0.26	26
	(0.38%)	(0.25%)	(0.10%)	(12.0%)

Transport activity

The transport activity of the fractions after incorporation into liposomes is given in Table I. Sodium-dependent D-glucose transport increased from 225 pmol/30 s per mg protein in proteoliposomes formed with the crude membrane extract to 6350 pmol/30 s per mg protein in proteoliposomes formed with the purified fraction. The protein fraction not retained by the phlorizin polymer showed not transport activity.

The time course of total D-glucose uptake and sodium-dependent D-glucose uptake by the proteoliposomes is given in Fig. 4. In comparing the glucose uptake by proteoliposomes formed with the crude membrane extract with the uptake by proteoliposomes formed with the purified fraction, two phenomena can be observed (Fig. 4A). First, the initial rate of D-glucose uptake is much faster in the proteoliposomes formed with the purified fraction and second, the uptake after 90 min of incubation is much higher. The stimulation of initial glucose uptake is more evident for the sodium-dependent part than for the sodium-independent part. As shown in Fig. 4B, a striking increase in transport activity occurs during purification. Sodium-dependent glucose uptake by the purified fraction is initially, when the sodium gradient exerts its full driving force, highest and then approaches zero.

In other experiments (data not shown) it was found that uptake of glucose at equilibrium decreases when the osmolality of the incubation medium is increased by the addition of sucrose. This indicates that uptake of glucose by proteoliposomes represents transport across the membrane into an osmotically reactive space and not merely binding to the liposomes.

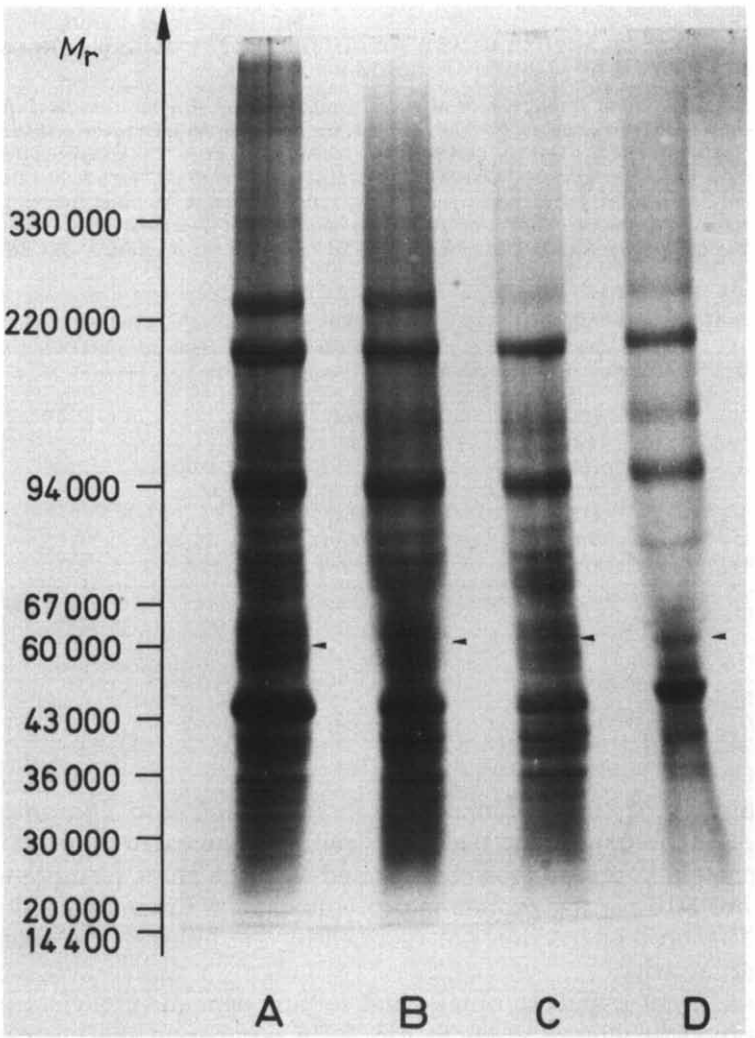


Fig. 3. SDS-polyacrylamide slab gels (4—30% acrylamide) with 0.2% SDS. (A) Brush border membrane; (B) fraction AS-I-0; (C) fraction AS-I-1; (D) fraction AS-I-2. For each gel, 80 µg protein were applied to the gel. Thyroglobulin (330 000 daltons) ferritin (220 000 daltons), albumin (67 000 daltons), phosphorylase A (94 000 daltons), albumin (43 000 daltons), carbonic anhydrase (30 000 daltons), trypsin inhibitor (20 100 daltons), and lactalbumin (14 400 daltons) were used as reference proteins.

Influence of phospholipid-to-protein ratio on protein incorporation and transport activity

In Table II, further information is provided on the proteoliposome system used in this study. Concerning the amount of protein incorporated under various conditions, 65% of the protein from the crude membrane extract is incorporated and 53% from the purified fraction. No difference in protein incorporation is observed when the amount of phospholipids is increased. In Table II, the sodium-dependent and sodium-independent glucose uptake using different lipid-to-protein ratios is given. An increase in lipid from 20 to 40 mg per

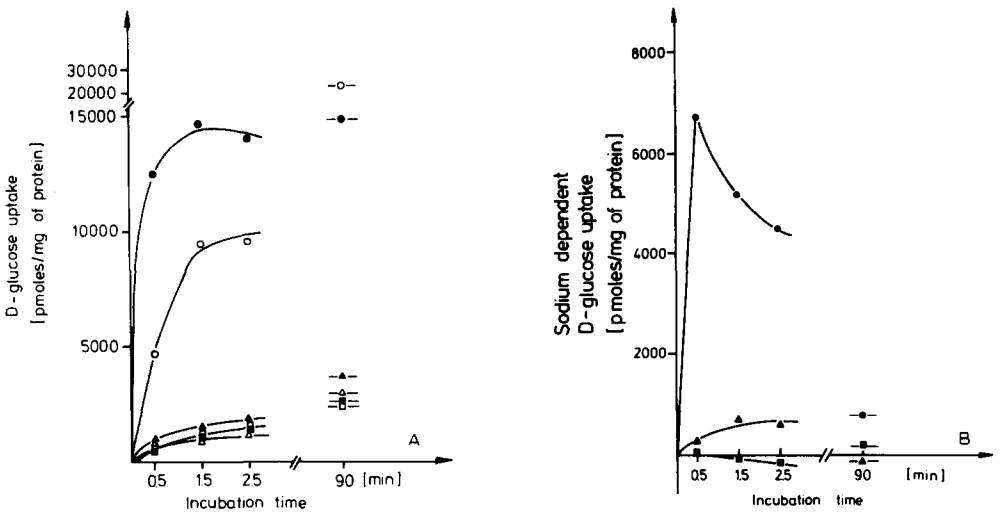


Fig. 4 (A) Time course of D-glucose transport by proteoliposomes. Proteoliposomes were prepared from asolectin and the crude membrane extract AS-I-0 (▲———▲), the protein fraction not retained by the phlorizin polymer AS-I-1 (■———■), or the purified fraction AS-I-2 (●———●). The liposomes contained 150 mM KCl, 20 mM Hepes-Tris, pH 7.4, 0.2 mM dithiothreitol, and 0.5 mM EDTA; liposomes were incubated in solutions containing in addition 0.2 mM D-[^{3}H]glucose and 150 mM KCl (open symbols) or 150 NaSCN (closed symbols). One representative experiment is given, the lipid-to-protein ratio varied from 34 : 1 to 46 : 1 (w/w). (B) Time course of sodium-dependent D-glucose transport by proteoliposomes. The values represent the difference between D-glucose uptake in the presence of NaSCN and KCl. Crude membrane extract AS-I-0 (▲———▲), protein fraction not retained by the phlorizin polymer AS-I-1 (■———■), and purified fraction AS-I-2 (●———●). Conditions as described in A.

mg of protein causes an increase in sodium-dependent glucose uptake by 85% proteoliposomes formed from the crude membrane extract and of 55% in proteoliposomes formed from the purified fraction. It is noteworthy that the degree of enrichment of sodium-dependent D-glucose transport activity in the purified fraction compared to the crude membrane extract is only slightly effected by the lipid-to-protein ratio. With a ratio of 20 : 1 the transport activity is 31-fold higher, with a ratio of 40 : 1 the transport activity is 26-fold higher.

TABLE II

INFLUENCE OF LIPID-TO-PROTEIN RATIO ON D-GLUCOSE UPTAKE BY PROTEOLIPOSOMES

D-Glucose uptake is given in pmol/mg protein per 30 s, the protein quantity used for calculation represents the incorporated protein. Enrichment was calculated as the ratio of the D-glucose uptake of fractions AS-I-1 and AS-I-2 to the starting fraction AS-I-0.

Fraction	Lipid/protein (mg/mg)	Uptake	Enrichment	Lipid/protein (mg/mg)	Uptake	Enrichment
Sodium-dependent uptake						
AS-I-0	20	160	1	40	300	1
AS-I-1	20	—92	~0	40	9	~0
AS-I-2	20	5000	31	40	7700	26
Sodium-independent uptake						
AS-I-0	20	407	1	40	740	1
AS-I-1	20	436	1	40	614	0.8
AS-I-2	20	1721	4.2	40	4729	6.4

The sodium-independent glucose uptake in the presence of KCl is identical in proteoliposomes formed from the crude extract or from the fraction not retained by the polymer. The uptake increases 4-fold (lipid-to-protein ratio 20 : 1) or 6-fold (lipid-to-protein ratio 40 : 1) when the purified fraction is used.

Discussion

Interaction of the sodium-D-glucose cotransport system with the phlorizin polymer

The results presented above indicate that the phlorizin polymer, which has been shown previously to bind specifically sugar binding proteins such as concanavalin A [8], also interacts with the sodium-D-glucose cotransport system extracted from renal brush border membranes. Evidence for this conclusion is derived from the following observations. (1) The transport activity found in the crude membrane extract is removed from the extract by incubation with the phlorizin polymer and (2) transport activity is recovered in a protein fraction eluted from the polymer with 0.5 M D-glucose. The first finding could also be explained by an inactivation of the transport system, lack of incorporation, or by a permeability of the proteoliposomes which makes the demonstration of sodium cotransport systems impossible. The two former arguments cannot be ruled out at the moment, the latter is improbable because sodium-dependent L-alanine transport can be demonstrated in the liposomes (unpublished observation). The recovery of transport activity after elution with 0.5 M D-glucose very strongly supports the assumption of an interaction of the glucose transport system with the polymer.

The nature of the interaction is probably quite complex. Firstly, an interaction with the D-glucose ligands which still retain the free conformational motions can be assumed. The concentration of glucose ligands accessible for sugar binding proteins is approx. 0.5 mM [8]. In view of the apparent K_m value of 1 mM D-glucose for the transport system, this interaction alone can hardly explain the strong binding of the glucose transport system to the polymer. An additional binding force has therefore to be assumed. This force is probably related to the hydrophobic aglucone moiety of phlorizin. Although used as a matrix for the polymerisation, the aglucone moiety might still affect the binding of the D-glucose transport system to the polymer. The maximum effect on transport system-ligand interaction elicited by the aglucone is manifested in the high affinity of the glucose transport system for free phlorizin; the affinity is approx. 1000-fold higher than the affinity for D-glucose alone. The affinity of the glucose transport system for the polymer is probably lower than its affinity for phlorizin, since the phlorizin molecule is immobilized. On the other hand, a hydrophobic residue at C1 of the glucose molecule increases the affinity [7], therefore a binding constant of between 10^{-5} and 10^{-4} M for the interaction between the glucose transport system and the polymer is most likely.

The hydrophobic nature of the gel, which favors the interaction of the glucose transport system with the polymer, however, also has disadvantages. Other hydrophobic membrane proteins are also retained and elute together

with the transport protein. This is evident from the complex protein pattern of the purified fraction.

Degree of purification of the D-glucose transport system

In the studies reported above, purification was assessed from the transport activities of proteoliposomes formed under constant lipid-to-protein ratios out of asolectin and the various fractions. This method has several drawbacks. These are related to the degree of inactivation, to the extent of incorporation and the orientation of the transport system within the liposomes and to the permeability properties of the liposomal membrane. In order to circumvent these inherent problems at least partially, we performed the incorporation with two different phospholipid-to-protein ratios and obtained similar increase in transport activity compared to the crude membrane extract. This increase is the highest reported so far in the literature. The actual degree of purification has to be determined in further investigations; phlorizin binding studies and transport studies, preferably on tracer exchange, performed in parallel should be most useful in this context. An interesting, however, as yet unexplained finding is the increase in sodium-independent transport and in the equilibrium uptake of the proteoliposomes formed with the purified fraction. If one interprets the former as simple diffusion, the increase in permeability might indicate a higher fluidity of the liposomal membranes. The cause of this increase might be residual detergent or the change in protein composition incorporated. It might also be possible that parts of the sodium-glucose transport system are uncoupled, i.e., have lost their sodium dependence and facilitate sodium-independent glucose transport. The increase in equilibrium value probably indicates a higher intravesicular space, however, no direct evidence for these assumptions is available.

It is clear from the protein pattern on SDS-polyacrylamide gels that the purified fraction contains more than one protein, therefore, only limited statements can be made about the identity of a protein band with the D-glucose transport system. If, however, the relative intensities of the protein bands in the crude extract and in the purified fraction are compared, one band with an apparent molecular weight of 60 000—70 000 seems to be particularly enriched in the purified fraction. If one assumes that this protein band represents the glucose transport system, the apparent molecular weight would be close to the molecular weight of the glucose transport system as determined in labeling experiments with *N*-ethylmaleimide or *p*-chloromercuribenzenesulfonic acid [15—18] or in the negative extraction experiments on the intestine [5].

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References

1 Kinne, R. and Faust, R.G. (1978) Biochem. J. 168, 311—314
2 Crane, R.K., Malathi, P. and Preiser, H. (1976) Biochem. Biophys. Res. Commun. 71, 1010—1016
3 Crane, R.K., Malathi, P., Preiser, H. and Fairclough, P. (1978) Am. J. Physiol. 234, E1—E5
4 Fairclough, P., Malathi, P., Preiser, H. and Crane, R.K. (1979) Biochim. Biophys. Acta 553, 295—306
5 Klip, A., Grinstein, S. and Semenza, G. (1979) J. Membrane Biol. 51, 47—53
6 Vannier, C., Louvard, D., Maroux, S. and Desnuelle, P. (1976) Biochim. Biophys. Acta 455, 185–199
7 Riedel, S. (1980) Dissertation, Rhein. Westf. Technische Hochschule, Aachen
8 Lin, J.T. and Kinne, R. (1980) Angew. Chem. 92, 547—548
9 Lin, J.T., Riedel, S. and Kinne, R. (1979) Biochim. Biophys. Acta 557, 179—187
10 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
11 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375—378
12 Book, F., Baumann, K. and Kinne, R. (1976) Biochim. Biophys. Acta 433, 294—310
13 Haase, W., Schäfer, A., Murer, H. and Kinne, R. (1978) Biochem. J. 172, 57—62
14 Kinne, R. (1976) Curr. Top. Membranes Transp. 8, 209—267
15 Lemaire, J. and Maestracci (1978) Can. J. Physiol. Pharmacol. 56, 760—770
16 Poiree, J.C., Mengual, R. and Sudaka, P. (1979) Biochem. Biophys. Res. Commun. 90, 1387—1392
17 Smith, M.W., Ferguson, D.R. and Buston, K.A. (1975) Biochem. J. 147, 617—619
18 Thomas, L. (1973) Biochim. Biophys. Acta 291, 454—464
19 Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477—5487